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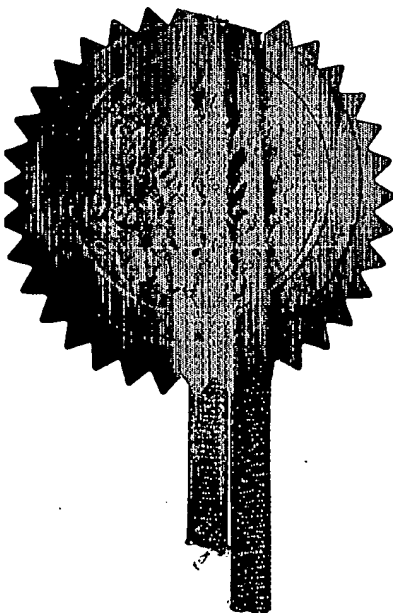
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P. Mahoney

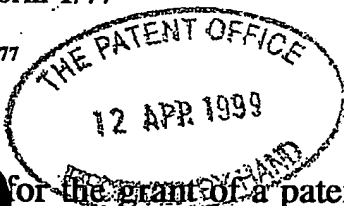
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1. Your reference

REP06110GB

2. Patent application number

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3. Full name, address and postcode of the or of
each applicant (underline all surnames)

Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the
country/state of its incorporation

GB

730454600/

4. Title of the invention

PURINE NUCLEOSIDE PHOSPHATASE AND
COMPOSITIONS CONTAINING IT

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom
to which all correspondence should be sent
(including the postcode)

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number (if you know it)

745002

6. If you are declaring priority from one or more
earlier patent applications, give the country
and the date of filing of the or of each of these
earlier applications and (if you know it) the or
each application number

Country

Priority application number
(if you know it)

Date of filing
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7. If this application is divided or otherwise
derived from an earlier UK application,
give the number and the filing date of
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Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right
to grant of a patent required in support of
this request? (Answer 'Yes' if:

YES

- a) any applicant named in part 3 is not an inventor
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Description 7

Claim(s) 2

Abstract

Drawing(s) 2 + 2

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Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date

12 April 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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PURINE NUCLEOSIDE PHOSPHATASE AND COMPOSITIONS CONTAINING

IT

Field of the Invention

This invention relates to one protein, to vaccines
5 containing it, and to its use in therapy, for
immunisation.

Background to the Invention

Group B Streptococcus (GBS), also known as
Streptococcus agalactiae, is the causative agent of
10 various conditions. In particular, GBS causes:

Early onset neonatal infection.

This infection usually begins *in utero* and causes
severe septicaemia and pneumonia in infants, which is
lethal if untreated and even with treatment is associated
15 with a 10-20% mortality rate.

Late onset neonatal infection.

This infection occurs in the period shortly after
birth until about 3 months of age. It causes a
septicaemia, which is complicated by meningitis in 90% of
20 cases. Other focal infections also occur including
osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Adult infections.

These appear to be increasingly common and occur
25 most frequently in women who have just delivered a baby,
the elderly and the immunocompromised. They are
characterised by septicaemia and focal infections
including osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

30 *Urinary tract infections.*

GBS is a cause of urinary tract infections and in
pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn, leads to reduced milk production and is therefore considerable economic importance.

5 GBS infections can be treated with antibiotics. However, immunisation is preferable. It is therefore desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

10 According to the present invention, a partial GBS gene sequence for the protein Purine Nucleoside Phosphatase (PNP) has been found which represents an outer surface protein.

15 In one aspect of the invention, the use of this protein in a recombinant protein vaccine is described. This vaccine may be administered to females either prior to, or during pregnancy to protect mother and neonate against infection by GBS.

20 The gene sequence may be first genetically altered to increase the antigenicity of the encoded protein.

Brief Description of the Drawings

The invention will now be described in detail with reference to the accompanying figures, wherein:

25 Figure 1 shows the partial nucleotide sequence and the deduced amino acid sequence of the GBS PNP.

Figure 2a shows peptide sequence of the PNP obtained from MS/MS peptide sequencing

30 Figure 2b shows the sequence of oligonucleotides derived from the sequences in 2a used for PCR amplification of GBS PNP.

Description of the Invention

Because of its extracellular or cell surface location, the protein of the present invention may be a

suitable candidate for the production of therapeutically-effective vaccines against GBS. The term "therapeutically-effective" is intended to include the prophylactic effect of the vaccines. For example, a
5 recombinant protein may be used, as an antigen for direct administration to an individual. The protein may be isolated directly from GBS or expressed in any suitable expression system, e.g. *Lactococcus lactis*. It is preferably administered with an adjuvant, e.g. alum.

10 The protein may be a mutant protein in comparison to wild-type protein, a fragment of the protein or a combination of different fragments, provided an effective immune response is generated.

An alternative approach is to use a live attenuated
15 GBS vaccine. This may be produced by deleting the gene that encodes the protein. Preferably, the GBS strain comprises additional virulence gene mutations.

The protein (or fragments thereof) of the present invention may also be used to produce monoclonal and
20 polyclonal antibodies for use in passive immunisation.

In a further embodiment of the invention, the protein or corresponding polynucleotide may be used as a target for screening potentially useful drugs, especially antimicrobials. Suitable drugs may be selected for their
25 ability to bind to the protein to exert their effects. Assays for screening for suitable drugs and which make use of the protein of the invention will be apparent to those skilled in the art.

Although the protein has been described for use in
30 the treatment of individuals, veterinary uses of the protein are also considered to be within the scope of the present invention. In particular, the protein or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

The present invention is described with reference to Group B Streptococcal strain M732. However, all the GBS strains and many other bacterial strains are likely include related proteins having amino acid sequence homology with the protein of M732. Organisms likely to contain the proteins include, but are not limited to, *S. pneumoniae*, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G Streptococci and Enterococci. Vaccines to each of these may be developed in the same way as described for GBS.

Preferably, the proteins that may be useful for the production of vaccines have greater than 40% sequence similarity with the protein of M732. More preferably, the proteins have greater than 60% sequence similarity. Most preferably, the proteins have greater than 80% sequence similarity.

The protein of the present invention was identified as follows:

Todd-Hewitt Broth was inoculated with GBS and allowed to grow overnight at 37°C. The cells were harvested by centrifugation and washed with Phosphate Buffered Saline (PBS). The cells were resuspended in an osmotic buffer (20% (w/v) Sucrose, 20mM Tris-HCl pH 7.0, 10mM MgCl₂) containing protease inhibitors (1 mM PMSF, 10 μM Iodoacetic Acid, 10 mM 1,10-Phenanthroline, 1 μM Pepstatin A) and Mutanolysin at a final concentration of 4 Units per microlitre. This was incubated (shaking) at 37°C for 2 hours.

Cells and debris were removed first by high speed centrifugation, then ultra-centrifugation for 1 hour. The resultant supernatant containing cell wall proteins was concentrated under pressure using an ultrafiltration device (10,000 molecular weight cut-off).

The sample was dialysed against ultra high quality water and lyophilised. After resuspension in loading buffer, the proteins were separated by preparative 2-Dimensional-Gel Electrophoresis. Following

5 Electrophoresis an individual spot was chosen for study. The spot was subjected to in-gel tryptic digestion. The resulting peptides were extracted from the gel and purified using microbore RP-HPLC. Fractions were collected every 45 seconds and a portion of these

10 consistent with the regions of UV absorbance were analysed by Delayed Extraction-Matrix Assisted Laser Desorption-Time of Flight Mass Spectrometry (DE-MALDI-TOF-MS). Peptides not observed in a blank preparation were then subjected to sequencing using Nanospray-MS/MS

15 The Peptide Sequences obtained are shown in Figure 2a.

Using this information, degenerate oligonucleotides were designed to be used in a polymerase chain reaction (PCR) to amplify the DNA segment lying between the

20 peptide sequences identified. The sequences of these oligonucleotides is shown in Figure 2b.

PCR amplification resulted in the production of an (approximately) 230 base pair fragment, which was cloned into the pCR 2.1-TOPO vector (Invitrogen BV, Netherlands)

25 according to manufacturers protocol. This plasmid was termed pMS14. The cloned DNA fragment was sequenced (Figure 1). The deduced amino acid sequence was used to search protein databases. Results of this search are shown in Table 1.

30 As shown in Table 1, homologues to the GBS MS14 gene product can be identified in *Bacillus subtilis*, *Bacillus stearothermophilus*, *Mus musculus*, *Bos taurus* and *Zea mays*. In all cases the homologues are the genes for the protein Purine Nucleoside Phosphatase (PNP). The

function of this enzyme is to cleave the nucleosides
guanosine or inosine to their respective bases and sugar
1-phosphate molecules in the presence of orthophosphate

Table 1. Database search results for MS10

Organism	Protein Accession	DNA Accession	Gene Name	% Similarity	% Identity	Alignment Length
<i>Bacillus subtilis</i>	P46354	U32685	Purine Nucleoside Phosphatase	81	67	73
<i>Bacillus stearothermophilus</i>	P77834	D87959	Purine Nucleoside Phosphatase	75	58	77
<i>Mus musculus</i>	P23492	X56548	Purine Nucleoside Phosphatase	73	59	74
<i>Bos taurus</i>	P55859		Purine Nucleoside Phosphatase NADP-	71	61	74

CLAIMS

1. A surface-associated solute-binding protein obtainable from a Group B streptococcal strain.
2. A protein according to claim 1, obtainable from the
5 Group B streptococcal strain M732.
3. A protein according to claim 2, encoded by the polynucleotide defined as MS14 in Figure 1 or a mutant thereof.
4. A protein according to claim 3, wherein MS14
10 comprises the nucleotides.1-242
5. A protein according to any of claims 1 to 4, for use in a method of therapy.
6. A protein according to claim 5, for use in the treatment of GBS infection.
- 15 7. A polynucleotide which encodes a protein according to any preceding claim.
8. The use of a bacterial protein having amino acid sequence similarity with a protein according to any of claims 1 to 6 and which is surface associated, in
20 the manufacture of a vaccine to treat bacterial infection.
9. The use according to claim 8, wherein the infection is a Group B streptococcal infection.
10. The use according to claim 8 or claim 9, wherein the
25 infection is a focal infection.
11. The use according to claim 8 or claim 9, wherein the infection is a urinary tract infection.
12. Use of a product according to any of claims 1 to 7, for screening potential antimicrobial drugs.
- 30 13. An antimicrobial drug selected using the products as defined in claim 12.
14. A vaccine comprising a protein according to any of claims 1 to 6 and 8.

15. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene codes for a protein according to claim 8.
16. An antibody raised against a protein according to any of claims 1 to 4.

Figure 1. Nucleotide and deduced amino acid
sequence of clone MS14

10	30	50
TTTCATTTTTACGAAGGTAATACAATGGAAGTCGTTACTTTCCCAGTACG		
F H F Y E G N T M E V V T F P V R		
70	90	
TATCATGAGAGCATTGGCTTGCCACAGTGTGCTTGTGACTAATGCAGCGG		
I M R A L A C H S V L V T N A A G		
110	130	150
GTGGGATTGGATACGGACCAGGAACCTTTAATGCTGATCAAAGGCCACATC		
G I G Y G P G T L M L I K G H I		
170	190	
AATATGATTGGGACTAACCCTCTCATAGGTGAGAACCTTGAAGAATTTGG		
N M I G T N P L I G E N L E E F G		
210	230	
ACCACGTTTTCCAGACATGTCGGATGCTTACACAGCAACATA		
P R F P D M S D A Y T A T		

Figure 2a. Generated Peptide Sequences for MS14

FHFYEGNT

DAYTATYR

Figure 2b. Oligonucleotide sequences designed from
Peptide sequences in Figure 2a

TTYCATTTYTAYGAAGGWAATAC

RTAWGTWGCWGTRTAWGCATC

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13. An antimicrobial drug selected using the products as defined in claim 12.
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      70              90
TATCATGAGAGCATTGGCTTGCCACAGTGTGCTTGTGACTAATGCAGCGG
I M R A L A C H S V L V T N A A G

     110              130              150
GTGGGATTGGATACGGACCAGGAACCTTTAATGCTGATCAAAGGCCACATC
G I G Y G P G T L M L I K G H I

           170              190
AATATGATTGGGACTAACCCTCTCATAGGTGAGAACCTTGAAGAATTTGG
N M I G T N P L I G E N L E E F G

     210              230
ACCACGTTTCCCAGACATGTCGGATGCTTACACAGCAACATA
P R F P D M S D A Y T A T
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Figure 2b. Oligonucleotide sequences designed from
Peptide sequences in Figure 2a

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RTAWGTWGCWGTRTAWGCATC

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